Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(2): 97-99 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Extraction, Isolation and Antimicrobial Activity of Crude and Purified Ferritin extract from seeds of Soyabean (*Glycine max* (L.) Merr.)

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Abstract: The present study aimed to isolate, purify and evaluate the antimicrobial activity of ferritin extracted from Soyabean (*Glycine max (L.) Merr.*) against Gram-negative microorganisms (*Escherichia coli, Pseudomonas aeruginosa, Kliebsiella Pneumonia, Proteus vulgaris*), as well as Gram-positive microorganism (*Staphylococcus aureus, Staphylococcus epidermis*) and fungus *Candida albicans.* Agar well diffusion method was adapted to determine antibacterial activity against all the test microorganisms. Zone of inhibition of the crude and pure extracts were tested. Among all the test pathogens *E. coli* was found susceptible with zone of inhibition 9mm. to the crude and purified ferritin extract. The present study successfully isolated and purified the single unit of *Glycine max* (L.) Merr**.** ferritin with 28-kDa units . It also reveals the least susceptibility of the microorganisms towards the ferritin isolated from seeds of *Glycine max (L.) Merr.*
Keywords: Ferritin, *Glycine max (L.) Merr.* , Antimicrobial activity

INTRODUCTION

For thousands of years natural products have played a very important role in healthcare and prevention of diseases. The ancient civilizations of the Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases [1]. However, it was not until the nineteenth century that scientists isolated active components from various medicinal plants. According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine [2]. About121 drugs prescribed in USA today come from natural sources, 90 of which come either directly or indirectly from plant sources [3].

Medicinal plants have continued to attract attention in the global search for effective antimicrobial agents that can combat resistant pathogens that have been rendering many conventional drugs obsolete in the treatment of infections [4].

The increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some bacterial diseases and hence research for identifying novel substances that are active against human pathogens is an urgent need [5].The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [6]. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resist antimicrobial pathogens. However, very little information is available on such activity of medicinal plants [7, 8].

Soy contains significant amounts of all the essential amino acids for humans, and so is a good source of protein, which is generally used to prepare extracts or powders for medicinal use. The current investigation aims to explore scientifically the antimicrobial potential of crude and pure ferritin seed extract of *Glycine max (L.) Merr.* plant.

MATERIALS AND METHODS

Chemicals:

Phenylmethylsulfonyl fluoride (PMSF) was obtained from Sigma-Aldrich Chemicals Pvt Limited. $MgCl_2$ and other chemicals used for extractions were of HPLC grade and were purchased from M/S Merck ltd. Mumbai.

Plant Materials

Seeds of *Glycine max* (L.) Merr. were collected from Shivamogga district of Karnataka state. The collected seeds were washed and shade dried. The

dried seeds were disinfected with 15%H_2O_2 and stored at room temperature in sterile sealed bottled until its extraction.

Source and Maintenance of Test Microorganisms

The bacterial organisms included in the study were obtained from National Chemical Laboratory (NCL), Pune, India. Gram positive (*S. aureus, S. epidermidis*) and Gram Negative (*E. coli, K. pneumoniae , P. vulgaris*, and *P. aeruginosa*) and Fungal (*C. albicans*) were included for antimicrobial evaluation. All the bacterial microorganisms were maintained on nutrient agar, while the fungal strain was maintained on potato dextrose agar slope at 4 °C and were sub cultured at frequent intervals as required. Twenty-four hour old pure cultures were prepared for antimicrobial analysis of the each test sample.

Extraction and Isolation of Plant Ferritin

500g of seeds *of Glycine max (L.) Merr.* were washed and shade dried. 500g of seeds were soaked in flat bottomed trays in 3 volumes of double deionized water. Soaking was carried out for 72 hrs. Homogenization was performed in a Waring Blender set on high for 60secs in 2 volumes of double deionized water made to 0.1mg/ml in phenylmethylsulfonyl fluoride (PMSF). The slurry was filtered through 4 layers of cheesecloth and centrifuged at 12000 X g for 15 min. The precipitate was dried and labelled as a crude ferritin. Same filtrate was precipitated with $MgCl_2$, the precipitate is dried and labelled as purified ferritin. The final protein powder crude were dissolved in sterile buffer and used for antimicrobial studies [9].

Purification and Protein Gel Electrophoresis

Following the ribosomal-ferritin precipitation by $MgCl_2$, the centrifuged pellet was redissolved in 0.01M $NaPO_4$/HCl, PH 7.4(PO_4 buffer) and dialyzed against 3 x 6 litres of the buffer. The supernatant was concentrated with ammonium sulphate (50%) and dialyzed in the same buffer. The final product so obtained was labelled as pure ferritin and used for antimicrobial studies. Purity of the preparation was assessed by gel electrophoresis in a nondenaturing system with 12.5% polyacrylamide SDS gel as described earlier.

Polyacrylamide gradient gel calibrated with horse spleen ferritin (Sigma-Aldrich Chemicals Pvt Limited) was used to determine the molecular mass of the (*Glycine max* (L.) Merr**.)** seed ferritin oligomer [10,11].

Antimicrobial Susceptibility Test:

In vitro antibacterial activities of the crude extracts of ferritin from three different sources were studied against Gram-negative and positive bacteria and a fungal strain by the agar well diffusion method [12]. The extracts were dissolved in 0.01 M $NaP0_4$/HC1, pH 7.4 (PO_4, buffer) to a final concentration of 10%. Nutrient Agar (Himedia, Mumbai) was used as the bacteriological medium and Potato's dextrose agar for fungal microorganism. Agar medium was autoclaved and dispensed at 20 ml per plate in 12 x 12 cm petri dishes. Suspension of micro-organisms was made in sterile saline water. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. A sterile cork borer of 5mm diameter was used to make wells on the medium. 50µl of the three crude extracts were dispense into each labelled well [13, 14]. The nutrient Agar plates were incubated at 37^0C for 24 hrs and Fungal plates were incubate at room temperature for 48 hrs. Sterile 0.01 M $NaP0_4$/HC1, pH 7.4 (PO_4, buffer) was taken as the negative control (Placebo) and Cephalothin & Itraconazole as the positive control. Antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. All the tests were carried out in triplicates and the final diameter of zone of inhibition was expressed as the average of the three readings.

Results and Discussion

Ferritin was isolated from the *Glycine max (L.) Merr.* seeds in crude and purified form. Ferritin can be isolated in a good yield, with approximately 35-38 mg of protein per 500g seeds. Electrophoresis on nondenaturing gels indicates that the ferritin is quite pure, with only a single band detected on a Coomassie-stained gel (Fig 1). Molecular mass analysis of the protein as compared with the standard essentially all the protein was of the 28- kDa form.

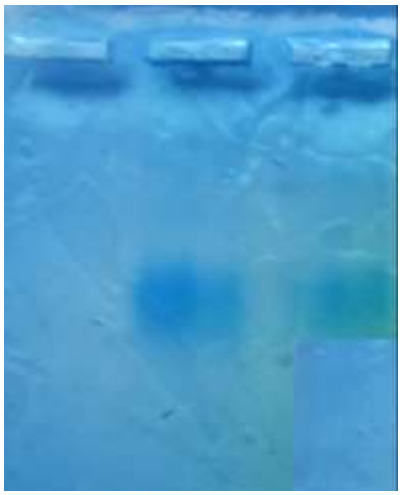

Fig1. Polyacrylamide gel electrophoresis of purified (*Glycine max* (L.) Merr.) ferritin.

The antimicrobial analysis of the crude and purified *Glycine max* (L.) Merr**.** ferritin showed the least potency against major pathogens. All the crude and purified ferritin from the seeds of the plant *Glycine max (L.) Merr.* tested showed no activity against most of the

pathogens .The least susceptibility was exhibited by the crude and purified extract of ferritin from *Glycine max (L.) Merr.* against *E. coli* with the average diameter of zone of inhibition as 9.11 mm (Table 1). All other microbial strains under the present investigation showed resistance towards both the crude and purified ferritin extracted from the seeds *Glycine max* (L.) Merr. plant respectively.

Table 1: Antimicrobial effect of crude and pure ferritin from Soyabean

Microorganisms	**Zone of Inhibition (mm)***	
	Crude *Ferritin*	***Pure Ferritin***
E. coli	Nil	9.11
K. pneumoniae	Nil	Nil
P. aeruginosa	Nil	Nil
P. vulgaris	Nil	Nil
S. aureus	Nil	Nil
S. epidermis	Nil	Nil
C. albicans	Nil	Nil

(*Average of the triplicate test results)

CONCLUSION

Traditionally plant parts, extracts and decoctions are used for treatment against microorganisms. Screening of in vitro antimicrobial activity of the medicinal plant involves various approaches which contribute for the different degree of susceptibility (Zone of inhibition) against different microorganisms [15, 16]. We selected to study ferritin from soyabean (*Glycine max* (L.) Merr**.)** seeds because these seeds are among the highest sources of iron found in plants [17]. We were quite successful in our attempts to isolate and purify the ferritin from soyabeans (*Glycine max* (L.) Merr**.)** . The present study demonstrate the first time the evaluation of the antimicrobial activity of crude and purified ferritin from the protein rich plant sources as soyabeans (*Glycine max* (L.) Merr**.)**. However, results reveal that the crude and purified ferritin from *Glycine max (L.) Merr.* seed may contribute for the antibacterial activity. The present study enables to demonstrate the antimicrobial activity of the phytoferritin obtained from soyabeans *(Glycine max (L.) Merr.)* successfully.

ACKNOWLEDGEMENT

The technical support and laboratory facility provided at Rishi Biotech is gratefully acknowledged.

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